

LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

The changes in the erythrocyte lipid peroxidation products (MDA), levels of glutathione (GSH), ascorbic acid and plasma vitamin E (non enzymatic antioxidant parameters) and activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GP_x), catalase in erythrocytes and plasma glutathione – S – transferase (GST) activity were estimated in patients with rheumatoid arthritis. This work was undertaken to assess oxidative stress and anti oxidant status in patients with rheumatoid arthritis. It was observed that there was a significant increase in erythrocyte MDA levels, activities of SOD, GP_x , plasma GST and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with rheumatoid arthritis when compared to controls. The results of our study suggests higher oxygen free radical production, evidenced by increased MDA and decreased GSH, ascorbic acid, vitamin E and Catalase activity, support to the oxidative stress in rheumatoid arthritis. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

KEY WORDS

Malondialdehyde, Glutathione, Ascorbic acid, Vitamin E, Superoxide dismutase, Catalase, Glutathione peroxidase, Glutathione -S - transferase, Rheumatoid arthritis.

INTRODUCTION

Arthritis, the joint inflammation, refers to a group of diseases that cause pain, swelling, stiffness and loss of motion in the joints. Rheumatoid Arthritis (RA) is a chronic, systemic disease, in which various joints in the body are inflamed, leading to swelling, pain, stiffness, and the possible loss of function. It is an autoimmune disease in which the body's immune system attacks itself. Rheumatoid Arthritis affects approximately 1-2% of the total world's population (1). Annual incidence rate of rheumatoid arthritis between 0.5%-1% of total population is reported every year in both developed and developing

countries (2). Lower incidences of rheumatoid arthritis are reported every year in East Asia (3). Rheumatoid Arthritis affects around 1 in 50 people and is more common in women than men. It is most common after the age of 40, but can happen at any age. Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues (4). The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies (5, 6). Alteration in the oxidant – antioxidant profile is known to occur in rheumatic diseases (7, 8). Oxidative stress due to damage brought about by free radicals is also known to influence the response of these patients to therapy. Moreover the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions (9) and two main categories of antioxidants are those whose role is to prevent the

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generation of free radicals and those that intercept any free radicals that are generated (10). They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes. The aim of our study was to investigate the changes in oxidant and antioxidant status in patients with rheumatoid arthritis.

MATERIALS AND METHODS

The study was conducted in Department of Biochemistry and Orthopaedics, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences & Research Foundation, Chinoutpally, Gannavaram (Mandal), A.P, India. Thirty clinically diagnosed patients from orthopaedics OPD, who had not undergone any previous treatment for their arthritis, were chosen for the study. Out of the thirty patients 12 were females. An equal number of age & sex matched healthy subjects with similar socio economic status were also investigated. Due permission was obtained from the ethical committee of the Dr.PSIMS&RF General Hospital, Chinoutpally before the start of the work. The written consents were also taken from the patients prior to study and the objectives of the study were fully explained. Nine of the participants were dropped out at the end of the selection, as they did not like the idea of giving blood.

The complete clinical and personal history of the subjects was recorded. The subjects were ranging in age 35 – 60 years. All the patients in the study were clinically diagnosed as patients with rheumatoid arthritis. The presence of rheumatoid arthritis in patients was diagnosed by carrying out X – ray analysis of joint destruction, rheumatoid factor test, C – reactive protein and antinuclear anti bodies test. Subjects with diabetes/systemic diseases like hypertension/diseases of any origin other than osteoarthritis which could cause oxidative stress or those receiving anti-inflammatory drugs in last 6 months were excluded. None of the participants was alcoholic or chronic smoker. Subjects with normal nutritional habits without supplementing any vitamins during last 6 months were included.

The heparinised venous blood samples obtained from these subjects were used for the analysis. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for the estimation of vitamin E and GST. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al (11), modified by Quist (12). The packed cells were used for the analysis of GSH, ascorbic acid, MDA, SOD, catalase, GP_X. Erythrocyte GSH was estimated by the method of Beutler et al (13) using

di thio bis nitro benzoic acid (DTNB). Ascorbic acid levels were estimated by the method of Tietz (14). Plasma vitamin E levels were estimated by the method of Baker H et al (15). MDA was determined as the measure of thio barbituric acid reactive substances (TBARS) (16). SOD (EC 1.15.1.1) activity was determined in the hemolysate by the method of Misra & Fridovich based on the inhibition of auto oxidation of epinephrine to adrenochrome at pH 10.2 (17). Catalase (EC 1.11.1.6) activity was measured by the method of Beers and Sizer (16). The activity of Glutathione Peroxidase (GP_X, EC 1.11.1.9) was measured as described by Paglia and Valentine (19) in erythrocytes and GST (EC 2.5.1.18) was measured by using 1-chloro-2, 4-dinitro benzene (CDNB) (20). Necessary care was taken during sample collection, storage and assay.

All reagents used were of analytical reagent grade. DTNB, CDNB and thio barbituric acid were obtained from sigma chemicals, St.Louis, MO. Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the student's 't' – test using the stat -view package. The data were expressed as Mean±SD. P < 0.05 was considered as significant.

RESULTS AND DISCUSSION

The Mean ± SD of erythrocyte GSH, ascorbic acid, MDA, SOD, Catalase, GP_X, plasma vitamin E and GST are described in the Table1. There was a statistically significant increase in the erythrocyte MDA levels in patients with rheumatoid arthritis compared to controls. The activities of erythrocyte antioxidant

Table1: Malondialdehyde (MDA), glutathione, ascorbic acid, vitamin E, superoxide dismutase (SOD), catalase, glutathione peroxidase (GP_X) and glutathione – S – transferase in controls and patients with rheumatoid arthritis.

Parameter	Group1 (controls) n=30	Group2 (Patients) n=30
Glutathione (mg/gm of Hb)	18.6±3.01	14.35±2.35 *
Ascorbic acid (mg/dl)	4.86±0.33	3.16±0.28 **
Vitamin E (µmoles/liter)	7.55±2.74	5.68±1.42 **
MDA (nmoles/gm of Hb)	4.62±0.58	5.63±0.82 ***
SOD (EU/gm of Hb)	1877.90 ± 532.67	2712.57± 497.79***
Catalase (nmoleH ₂ O ₂ /decomposed/mg protein/1min)	9.54 ± 0.13	6.91 ± 0.15 **
GP _X (u/gm of Hb)	16.91 ± 1.67	39.7 ± 2.01 **
GST(micromoles/dl of plasma)	13.65 ± 6.46	21.43 ± 6.97 ****

Values are mean ± SD

*P < 0.0001 compared to controls; ** P < 0.05 compared to controls

P< 0.001 compared to controls; *P < 0.01 compared to controls

enzymes SOD, GP_X and plasma GST were significantly increased in group2 compared to group1. The levels of erythrocyte GSH, ascorbic acid, plasma vitamin E and Catalase activity were significantly decreased in patients with rheumatoid arthritis compared to controls.

In the present study the lipid peroxidation product i.e. MDA levels have been increased significantly in erythrocytes of the patients with rheumatoid arthritis. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. Similar reports of elevated MDA levels have been reported in patients with rheumatic disease (7, 8). In contrast to our study, Kajanachumpol et al reported no significant change in MDA levels in patients with rheumatoid arthritis compared to controls (21).

We observed a significant decrease in the levels of erythrocyte GSH, ascorbic acid and plasma vitamin E (non enzymatic antioxidant defense system) in patients with rheumatoid arthritis when compared to controls. The decrease in the levels of these non-enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in Rheumatoid Arthritis. Similar reports of elevated MDA levels have been reported in patients with rheumatoid arthritis (22, 23).

In our study the erythrocyte antioxidant enzymes, i.e. SOD & GP_X activities have been increased significantly in patients with rheumatoid arthritis. Similar reports of raised SOD & GP_X activities have been reported in patients with rheumatoid arthritis (7, 24). SOD is the important antioxidant enzyme having an antitoxic effect against super oxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. GP_X, an oxidative stress inducible enzyme plays a significant role in the peroxy radical scavenging mechanism and in maintaining functional integration of the cell membranes (25). The rise in the activity of GP_X could be due to its induction to counter the effect of increased oxidative stress. Ostalowska et al have reported increased activities of SOD, glutathione peroxidase and glutathione reductase in synovial fluid of patients with primary and secondary rheumatoid arthritis of the knee joint (6).

The glutathione-S-transferases are a group of multifunctional proteins, which play a central role in detoxification of

electrophilic chemicals & the hepatic removal of potentially harmful hydrophobic compounds from blood (26). We have observed a significant rise in the activity of GST in patients with rheumatoid arthritis compared to controls. Similar reports of raised GST activity were observed in rheumatic diseases (27). The rise in the activity of GST could be due to its induction to counter the effect against increased oxidative stress.

In the present study, we have observed a significant decrease in the catalase activity in patients with rheumatoid arthritis compared to controls. Catalase is the enzyme, which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase (28). Similar reports of decreased catalase activity were observed in rheumatoid arthritis by Kerimova et al (29). However others have reported an increase in plasma catalase activity in patients with rheumatoid arthritis when compared to controls (7). In conclusion, Oxidative stress may be involved in rheumatoid arthritis. There is a shift in the oxidant – antioxidant balance in favour of lipid peroxidation, which could lead to the tissue damage observed in this disease.

REFERENCES

1. Deborah PM. Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. *Clin Rheum* 2002; 111: 172-7.
2. Gabriel SE. The epidemiology of rheumatoid arthritis. *Rheum Dis Clin North Am* 2001; 27: 269-81.
3. Shichikawa K, Inoue K, Hirota S, Maeda A, Ota H, Kilmura M, Ushiyama T, Tsujimoto M. Changes in the incidence and prevalence of rheumatoid arthritis. *Ann Rheum Dis* 1999; 58: 751-6.
4. Plaa GL, Witschi H. Chemicals, drugs and lipid peroxidation. *Ann Rev Pharmacol Toxicol* 1976; 16: 125-41.
5. Mapp PI, Grootveld MC. Hypoxia, oxidative stress and rheumatoid arthritis. *Br Med Bull* 1995; 51(2): 419-36.
6. Sato M, Miyazaki T. Antioxidants inhibit tumor necrosis factor-alpha mediated stimulation of interleukin-8, monocyte chemo attractant protein-1 and collagenase expression in cultured human synovial cells. *J Rheumatol* 1996; 23(3): 432-8.
7. Mezes M, Bartosiewicz G. Investigations on vitamin E and lipid peroxide status in rheumatic diseases. *Clin Rheumatol* 1983; 2(3): 259-63.
8. Ozkan Y, Yardym-Akaydin S, Sepici A, Keskin E, Sepici V, Simsek B. Oxidative status in rheumatoid arthritis. *Clin Rheumatol* 2006; 25: (Epub ahead of print).
9. Sie H. Oxidative stress: from basic research to clinical application. *Am J Med* 1991; 9: 31-8.

10. Cotgreave I, Moldeus P, Orrenius S. Host biochemical defense mechanisms against prooxidants. *Ann Rev Pharmacol Toxicol* 1988; 28: 189-212.
11. Dodge JF, Mitchell G, Hanahan DJ. The preparation and chemical characterization of hemoglobin free ghosts of human red blood cells. *Arch Biochem Biophys* 1968; 110: 119-30.
12. Quist EH. Regulation of erythrocyte membrane shape by calcium ion. *Biochem Biophys Res Commun* 1980; 92: 631-7.
13. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963; 61: 882-8.
14. Tietz N W. In; Text book of clinical chemistry, Edited by N W Tietz, W B Saunders company, Philadelphia, London, Toronto 1986; p. 960-2.
15. Baker H, Frank D, Winley N C. Clinical Vitaminology. 1968 ; p. 772.
16. Jain SK, Mcvie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. *Diabetes* 1989; 38: 1539-42.
17. Misra HP, Fridovich I. The role of super oxide anion in the auto oxidation of epinephrine and a simple assay for super oxide dismutase. *J Biol Chem* 1972; 247: 3170-5.
18. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by Catalase. *J Biol Chem* 1952; 195: 133-40.
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 158-9.
20. Warholm M, Guthenberg C, Christer von Bahr, Mannervik B. Glutathione transferases from human liver. In: Methods of enzymology, Alton Melster (Ed.). Academic Press 1985 ; 113: 500-01.
21. Kajanachumpol S, Vanichapunt M, Veraserntiyom O, Totemchokchyakarn K, Vatanasuk M. Levels of plasma lipid peroxide products and antioxidant status in rheumatoid arthritis. *Southeast Asian J Trop Med Public Health* 2000; 31(2): 335-8.
22. Jaswal S, Mehta HC, Sood AK, Kaur J. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. *Clin Chim Acta* 2003; 338(1-2): 123-9.
23. Kartas F, Ozates I, Canatan H, Halifeoglu I, Karatepe M, Colakt R. Antioxidant status and lipid peroxidation in patients with rheumatoid arthritis. *Ind J Med Res* 2003 ; 118: 178-81.
24. Cimen MY, Cimen OB, Kacmaz M, Ozturk HS, Yorgancioglu R, Durak I. Oxidant/antioxidant status of the erythrocytes from patients with rheumatoid arthritis. *Clin Rheumatol* 2000; 19(4): 275-7.
25. Chandra R, Aneja R, Rewal C, Konduri R, Dass K, Agarwal S. An opium alkaloid-papaverine ameliorates ethanol induced hepatotoxicity: diminution of oxidative stress. *Ind J Clin Biochem* 2000 ; 15(2): 155-60.
26. Smith GJ, Ohl VS, Litwack G, Ligandin. The Glutathione – S – Transferases, and chemically induced hepato carcinogenesis. A review, *Cancer Res* 1977; 37: 8-14.
27. Ostalowska A, Birkner E, Wiecha M, Kasperczyk S, Kasperczyk A, Kapolka D, Zon- Giebel A. Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary Rheumatoid Arthritis of the knee joint. *Rheumatoid Arthritis Cartilage* 2006; 14(2): 139-45.
28. Lenzi A, Cualosso F, Gandini L, Lombardo F, Dondero F. Placebo controlled double-blind cross over trial glutathione therapy, in male infertility. *Hum Reprod* 1993; 9: 2044.
29. Kerimova AA, Atalay M, Yusifov EY, Kuprin SP, Kerimov TM. Antioxidant enzymes;possible mechanism of gold compound treatment in rheumatoid arthritis. *Pathophysiology* 2000 ; 7(3): 209-13.